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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Francesco **Santangelo** Art Unit.: 1614

Serial No.: 10/583,334 Examiner: P.G. SPIVACK

Filed: March 7, 2007 Confirmation No.: 9753

For: **USE OF CYSTEIN OR CYSTEINE FOR THE PREVENTION AND TREATMENT OF OXIDATIVE STRESS CAUSED BY HAEMODIALYSIS AND OF ACUTE OR CHRONIC KIDNEY DISEASES**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION

I, Francesco Santangelo, declare as follows:

1 . My curriculum vitae is attached labeled as Exhibit 1.

2 I am the inventor of the subject matter of the present application.

3. An evaluation of the clinical efficacy of cysteine was carried out under my direction as follows:

For the demonstration of the eEcacy of oral cysteine in preventing oxidative stress, according to the use object of the present invention , the following randomized assessor-blind placebo-control prospective trial is followed.

All the patients, (30 in five groups) due to their state of chronic renal failure, were routinely submitted to 3 dialysis sessions lasting 4 hours, for at least three times a week. Cysteine by oral route has been administrated to four groups (one placebo control group) of patients at four increasing doses 500 mg, 600 mg, 800 mg and 1000 mg according the trial design reported below. Because it is useful to increase the Glutathione (GSH) level as soon as possible in order to fast counteract the oxidative stress related to the beginning of the extracorporeal circulation, a first administration has given just before the start of dialysis. A second administration was given just at the end of dialysis in order to restore the GSH consumed to counteract the overwhelming

oxidative stress caused by extracorporeal circulation. Also the placebo-control group of patients has the same characteristics. Such a treatment has been repeated for 4 weeks. A comparison between subjects under haemodialysis alone and under haemodialysis plus Cysteine has been performed. The treatment was open with assessor blind procedure. Each patients has been evaluated at the first dialysis procedure and after four weeks at the last planned dialysis procedure. A sample of whole blood has been drawn from all five groups just before the last dialysis session and analysed to evaluate the following two endpoints.

Efficacy assessment

To compare the effect of oral cysteine versus placebo the following parameters have been evaluated:

- The level of glutathione blood pellet as a marker of inflammation and oxidative stress.
- The level of haemoglobin concentration for the control of anaemia status.

Pilot study primary end point

From the evaluation of these parameters, the efficacy of cysteine (red blood cell glutathione concentration expressed as pg/ml) increased by around 9% at 500 mg, by around 15% at 600 mg, around 18% at 800 mg -and around 23% at 1000 mg - in the four treated groups resulting in lowering the oxidative stress and showing no effect on control group.

Secondary clinical endpoint

The decrease of anaemia due to escalatory Cysteine doses was assessed by measuring Haemoglobin concentration (g/l 00ml).

Increased Haemoglobin concentration at cysteine escalatory dosages of 500mg, 600mg, 800mg,

Increased Haemoglobin concentration at cysteine escalatory dosages of 500mg, 600mg, 800mg, 1000mg resulted in Haemoglobin concentration of 0,6 dl00 ml at 500 mg, 0,9 g/100ml at 600 mg, 1,1 g/100ml at 800 mg and of 1,3 g/100ml at 1000 mg (no increase in control group)

	Placebo	500 mg	600 mg	800 mg	1000 mg
haemoglobin increase	0g/ml	0.6g/ml	0.9g/ml	1.1 g/ml	1.3 g/ml
% increase red blood cell glutathione concentration	0%	9%	15%	18%	23%

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Milan March 1, 2003 Francesco Santangelo
Date Francesco Santangelo

Curriculum Vitae

Name: Francesco Santangelo

Birth: May 9th, 1953, Milan, Italy

Education: University - degree in Chemistry, Milan, Italy, July 1977 - (110/110)

University - degree in Pharmacy, Milan, Italy, November 1981 - (107/110)

Membership: American Chemical Society; European Free Radical Research Society; Society for Applied Pharmacological Science

Professional History

UNIVERSITY OF CAMERINO

Invited Professor at School of Pharmacy — from 2002

ZAMBON GROUP

Non-clinical Development Manager
International Project Manager — July 1997–June 2003

Head of Medicinal Chemistry Department: — October 1991 - June 1997

SIMES SPA
Head of Medicinal Chemistry Department — April 1982 - September 1991

FARMITALIA - CARLO ERBA
Medicinal Chemistry Laboratory Head: — June 1978- March 1982

MIDY / SANOFY MEDICINAL CHEMISTRY LABS
Medicinal Chemistry Laboratory Head: — December 1977 - May 1978

UNIVERSITY OF MILAN
Postdoctoral Fellow: — September 1977 - November 1997

Author of more than 75 scientific works and inventor in 54 patents applications most of them extended worldwide

Responsibilities from June 1997 till June 2003

Responsible for all activities of non-clinical development. Particularly, to prepare all the documentation need for the authorization of the first in man trial and all the other non-clinical studies to be included in registration dossier. The area of responsibility are chemical development, bioanalytical development, specific and safety pharmacology, toxicology and pharmacokinetic and metabolism. Moreover, he has managed some clinical pharmacology trial regarding the pharmacokinetic and disposition study.
At the same time, he has been the reference person for supporting N-acetylcysteine and, moreover, for all therapeutical proposals regarding new therapeutic application. In this respect, he has been responsible of a project of clinical development in tha area of Nephrology

List of publications since 1999

Papers:

1. J. Cortijo, M. Martinez-Losa, J. Jpiens, S. Esteve, F. Santangelo, L. Bruseghini, E. Morcillo
Inhibitory effects of N-acetylcysteine on superoxide anion generation by human eosinophils
Methods and Findings in Experimental and Clinical Pharmacology, 21, C08, **1999**
2. Morcillo E., Losa M., Santangelo F., Esteras A., et al
Preventive effect of oral N-acetycysteine on bleomycin-induced lung injury
Methods and Findings in Experimental and Clinical Pharmacology, 22, 418 (abs), **2000**
3. J. Cortijo, M. Cerdà-Nicolas, A. Serrano, G. Bioque, J. M. Estrela, F.Santangelo, A. Esteras, A. Llombart-Bosch, E. Morcillo
Preventive effect of oral N-acetycysteine on bleomycin-induced lung injury
Eur. J. Resp. **2001**, 17, 1228-1235
4. M. Napoletano, C. Fraire, F. Santangelo, E. Moriggi
Mesembrine is an inhibitor of PDE 4 enzyme which follows structure-activity relationship of Rolipram
The Chemistry preprint server (preprint.chemweb.com, Chemweb/medichem/0103001)
5. Julio Cortijo, Sebastian Blesa , M. Martinez-Losa, M. Mata, E. Seda , B.Sarriá, F. Santangelo E.J. Morcillo
Effects of taurine on pulmonary responses to antigen in sensitized Brown-Norway rats European Journal of Pharmacology, **2001,** 431, 111-117
6. F. Santangelo
The regulation of sulphurated amino acid junctions. Fact or fiction in the field of inflammation ?
Amino Acids, **2002,** 23, 359-365
7. Blesa S, Cortijo J, Seda E., Santamaria P., Santangelo F, Suchankova J, Morcillo.
Oral N-acetylcysteine attenuates the rat pulmonary inflammatory responses to antigen Methods and Findings in Experimental and Clinical Pharmacology, 24, Suppl A, 135, **2002**
8. Blesa S, Cortijo J, Martinez-Losa M, Mata M, Seda E, Santangelo F, Morcillo EJ.
Effectiveness of oral N -acetylcysteine in a rat experimental model of asthma.
Pharmacol Res **2002**, 45, 135-40
9. F.Santangelo
The modulation of the immune and inflammatory response by the metabolites of sulphur-containing aminoacids in Free Radicals, Nitric Oxide and Inflammation: Molecular, Biochemical and Clinical Aspects; Aldo Tomasi, Tomris Özben, Vladimir Skulachev, editors. Publisher: IOS-Press Amsterdam The Netherlands **2003,** 102-111
10. S. Blesa S., J. Cortijo , M. Martinez-Losa, M. Mata, E. Seda, F. Santangelo, E.J. Morcillo *Oral N-aceylcysteien attenuates the rat pulmonary inflammatory responses to antigen* Eur.Resp. J **2003**21, 394-400,
11. M Mata, A Ruíz, M Cerdá, J Cortijo, F Santangelo, A Serrano, A Llombart-Bosch, EJ Morcillo
Oral N-acetylcysteine reduces bleomycin-induced lung damage and mucin hypersecretion in rats
2003, Eur. Resp. J. *in press*
12. J Cortijo, M. Martinez-Losa, M. Mata, F. Santangelo, E.J. Morcillo.
Effects of N-acetylcysteine on functional responses of human eosinophils in vitro, **2003** *in press.*

13. F. Santangelo
Modulation of Inflammation by Regulation of Intracellular Thiol Level: an Approach to the Heart of Cell Functions.
Current Medicinal Chemistry **2003,** 10, :2599-610
14. F.Santangelo, J Cortijo, E.J. Morcillo
Taurine and the Lung. Which role in Ashma?
in Advances in Experimental Medicine and Biology, J. b: Lombarini, S. W. Sshaffer, J. Azuma eds,KLuwer Academic/Plenum Press, NY, USA, **2003**, vol 526, 403-410
15. L. Calvillo, S. Masson, M. Salio, L. Pollicino, N. De Angelis, A. Bai, P. Ghezzi, F. Santangelo* and R. Latini. (Istituto Mario Negri, *Zambon Group)
In vivo cardioprotection by N-acetylcysteine and isosorbide 5-mononitrate in a rat model of ischemia-reperfusion
Cardiovascular Drug and Therapy **2003**,17, 199-208
16. V. Witko-Sarsat, V. Gausson, A. Nguyen, M. Touam, T. Drueke, F.Santangelo* , B. Descamps
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Kidney International **2003,** 64, 82-96,.

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1. J. Cortijo, M. Martinez-Losa, J. Jpiens, S. Esteve, F. Santangelo, L. Bruseghini, E. Morcillo.
Inhibitory effects of N-acetylcysteine on superoxide anion generation by human eosiniphils
XI National Congress of the Spanish Society of Therapeutic Chemistry Valencia, Spain, September 14-17, **1999**
2. *F. Santangelo*
Tratamento Anti-oxidantes
XXX Congresso Brasileiro De Pneumologia e Tisiologia Gramado, Brasil, October 7-11, **2000**
3. F.Santangelo
Low flux oxygen therapy and oxidant modifications in COPD patients
Medical Forum Oxidative stress in different pathologies; new orizonts from N-Acetylcysteine Sevilla, October 20-21, **2000**
4. F.Santangelo
The regulation of sulphurated amino acid connection. A dummy or a protagonist on the stage of inflammation?
7^{Th} International Congress on Amino Acids and Proteins, Wein Austria August 6^{th} – 10^{th} , 2001
Proceeding in Amino Acids 21, 77-78, **2001**
5. F.Santangelo
The modulation of the immune and inflammatory response by the metabolites of sulphur-containing aminoacids
Free Radicals, Nitric Oxide and Inflammation: Molecular, Biochemical and Clinical Aspects Advanced Course sponsored by NATO, Federation of European Biochemical Society (FEBS) and Society Free Radical Research International, Antalya September 23^{rd} – October 3^{rd} , **2001**
6. Blesa S, Cortijo J, Mata M, Santangelo F, Suchankova J, Morcillo.
Oral N-acetylcysteine attenuates the rat pulmonary inflammatory responses to antigen National Congress of the Spanish Society of Pharmacology Toledo, Spain, September **2002**
7. F. Santangelo
Taurine and the Lung. Which role in Ashma?
International Taurine Symposium, Kauai, USA, September 20-23, **2002**
8. F.Santangelo
Antioxidant treatment. The central role of Glutathione in the modulation of inflammatory response.
12^{th} Russian Respiratory Congress, Moscow, Russia. 11-15- November, **2002**
9. F.Santangelo
The Regulation of Thiols and Glutathione as a Key Factor of Cell Life: Systemic Effect of N-Acetylcysteine on the Modulation of Inflammation
8^{Th} International Congress on Amino Acids and Proteins, Rome, Italy, 5-9 September, **2003**

Posters:

1. E. Morcillo, J. Cortijo, M. Mtnez-Losa, S. Blesa, M. Mata, F. Santangelo
   Effect of N-acetylcysteine on functional responses of human eosinophils in vitro
   American Thoracic Society Meeting, S. Francisco, Usa, May 19-23, **2001**
2. J. Cortijo, E. Morcillo, S. Blesa, M. Mata, J. Pelàez, F. Santangelo
   Effects of taurine on antigen-induced airway hyperrractivity, eosinophil accumulation, and microvascula leakage in sensitized Brown-Norway rats
   European Federation of Pharmacological Societies EPHAR, Lyon, France July 6-9, **2001**
3. E.Morcillo,J.Cortijo,M.Martinez-Losa, E. Seda, F. Santangelo
   Effects of taurine and taurine chloramine on functional responses of human eosinophils in vitro
   European Federation of Pharmacological Societies EPHAR, Lyon, France July 6-9, **2001**
4. R. Latini, L. Calvillo, S. Masson, M. Salio, L. Pollicino, N. De Angelis,, F. Santangelo*, (Istituto Mario Negri, *Zambon Group)
   In vivo Cardioprotection by N-acetylcysteine and isosorbide 5-mononitrate in a rat model of cardiac ischemia-reperfusion American College of Cardiology ACC, Chicago, Usa, March 30-April 2, 2003
5. F. Fiordaliso, R. Bianchi, L. Doni, T. Larangione, M. Salio, C. Savino, F. Santangelo*, S. Masson, P. Ghezzi, R. Latini (Istituto Mario Negri, *Zambon Group) *N-acetyl-Lcysteine attenuates cardiomyocyte death and reactive hypertrophy in streptocin-induced model of diabetes*
   American Heart Association Orlando, USA, 9-12 November, **2003**